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Meeting Report

# 7th Tuscany Retreat on Cancer Research: Genetic profiling, resistance mechanism and novel treatment concepts in cancer

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**7th Tuscany Retreat on Cancer Research.** Palazzo di Piero Chiusi, Tuscany, Italy, 6–13 August

For the seventh time, 60 scientists from 13 countries gathered in Palazzo di Piero, a medieval castle near Chiusi, Tuscany, Italy, to discuss the newest developments in the regulation of apoptosis, oncogenic signaling and aging with regard to cancer development and resistance mechanisms. The topics ranged from basic science to applied research in animal models and clinical trials of cancer research. Further scientific exchanges were inspired by the warm climate, excellent Italian cooking, delicious vino Montepulciano, refreshments in by the swimming pool and romantic outdoor dancing till the morning hours. As in previous years, the format of the conference strengthened existing collaborations, spurred new ideas and interactions between the participants and allowed young scientists to initiate or expand their future career network.

The meeting started with the introductory lecture by the organizer Christoph Borner (Freiburg, Germany) and the presentation of his group projects. He showed that the Semliki Forest virus induces apoptosis in infected cells by the BH3-only protein Puma and by initiating immunity signaling, which leads to the activation of caspase-3. Nina Raulf isolated new Bcl-$x_L$-binding partners by a proteomics approach. Andreas Geißler unraveled the JNK pathway as a mediator of apoptosis induced by gliotoxin, the major virulence factor of the fungus *Aspergillus fumigatus* that often strikes immunocompromised patients. Lars Joeckel characterized a signaling cascade that leads from the serine protease inhibitor AEBSF to ER-stress-mediated apoptosis. Karin Ferreira dissected the apoptotic pathways activated by Fas in mouse liver and in cultured hepatocytes. Whereas Fas ligand activates the mitochondrial type II pathway *in vivo*, it induces the type I pathway *in vitro*, underscoring the importance of animal studies in the apoptosis field.

Ulrich Maurer (Freiburg, Germany) introduced the scope of his laboratory's research, understanding of the function of GSK3$\beta$ and its substrates in apoptosis. Silke Lindner reported that a Mcl-1 mutant that is no longer targeted by GSK3$\beta$ promotes an increase in production of lymphocytes, as well as lymphoma development *in vivo*. Céline Charvet described another substrate of GSK3$\beta$: TIP60, an acetyltransferase that is critical for p53 to induce apoptosis. Martin Brandenburg presented interesting results on a less well-studied Bcl-2 family member, which may be important for the cell cycle checkpoint controlling M phase.

The overview of projects of Reuven Stein's laboratory (Tel-Aviv, Israel) was centered on mitochondrial dysfunctions in neurodegenerative diseases. Liora Lindenboim showed that upon apoptotic stimuli, nuclear proteins are redistributed to the cytoplasm in a caspase-independent manner, a process that requires the expression of Bax and Bak. This study will be extended by the use of a Bax-inducible cell model, described by Ayelet Amsalem. Another interest of Stein's laboratory is the ectoenzyme CD38, which has a role in microglial activation. Using the CD38 knockout mice model, Ayelet Levy revealed an interesting connection between microglia activation and tumor development. Her findings suggest that CD38 may be a promising drug target. Eran Blacher reported on the latest findings for a CD38 inhibitor that may be beneficial in various brain pathologies, such as Alzheimer's disease. Yulia Slobodskoy demonstrated a link between presenilin-1 and the master regulator of mitochondrial biogenesis PGC-1$\alpha$, thereby further connecting mitochondrial dysfunction and Alzheimer's disease.

Ana García-Sáez (Heidelberg, Germany) is interested in understanding how – at the biophysical level – the network of interactions between the Bcl-2 proteins controls the

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permeabilization of the mitochondrial membrane. Using giant unilamellar vesicles, recombinant fluorescently tagged proteins and biophysical measurements, she found that the membrane promotes complex formation between Bcl-2 proteins, and in doing so adds an important step in our understanding of the regulation of apoptosis.

Then, it was up to Gerry Melino's group (Leicester, UK) to present the p53 family members p73 and p63. Gerry Melino summarized recent findings in knockout animal models and suggested that one of the most important biological roles that the p53 family has evolved to fulfill is to preserve the integrity of the germ line, thereby guaranteeing reproductive fitness. Alessandro Rufini showed that depletion of p73, and specifically the TAp73 isoform, results in worsening of several aging parameters in mice and in cell lines. His data suggested that dysfunctional mitochondria and compromised metabolism may account for the increased oxidative load and for the accelerated aging triggered by depletion of TAp73. Tania Velletri used a neuronal differentiation cell culture model to determine that the TAp73 regulates expression of a mitochondrial protein that is important in neuronal differentiation, a process in which p73 has a fundamental role. Massimiliano Agostini showed that p73 directly drives the expression of a specific micro-RNA (miR) whose transcript levels are significantly reduced *in vivo* in the brains of p73 null mice and are correlated with TAp73 expression levels in cases of Alzheimer's patients. P73 also directly regulates expression of another neuronal gene: the nerve growth factor receptor ($p75^{NTR}$). Maria Victoria Niklison-Chirou showed that p73-mediated regulation of $p75^{NTR}$ sustains NGF-promoted axonal growth and branching. Francesca Grespi showed that mouse tissues express the vast majority of the p73 C-terminal isoforms already described in humans, and that this pattern of expression is modulated either during development or during chemotherapy treatment. Paola Tucci showed that p63 inhibits the migration of prostate cancer cells via regulation of expression of a specific miR. Interestingly, she found loss of p63 and the identified miR in human tumors, suggesting that it may be a useful clinical predictor of metastatic behavior. MiRs affect multiple biological processes, and Ivano Amelio showed that during keratinocyte differentiation, another miR target of p63 is upregulated and has a fundamental role in orchestrating the complex cytoskeleton remodeling that accompanies this process.

Mahvash Tavassoli (London, UK) is searching for proteins that will be therapeutically useful for treatment of head and neck cancers. One such protein is apoptin, a viral protein that selectively kills cancer cells. She showed that apoptin and TAp73 induce an E3 ligase that releases the inhibitory effects of a p73 isoform on cell death. PKC-$\beta$ is involved in apoptin-induced cell death in cancer cells. Jessica Bullenkamp presented the details of the PKC-$\beta$–apoptin interactions and the possible role of other kinases in the apoptin cell death-inducing capacities.

Ras proto-oncogenes were the major theme of the Kloog laboratory (Tel-Aviv, Israel) session. The Ras inhibitor FTS (S-trans,*trans*-farnesylthiosalicylic acid) was developed in the Kloog laboratory and is now undergoing clinical trials. Yoel Kloog presented the mechanism of action of this compound, suggesting that it competes with the farnesyl moiety of Ras on binding to a specific lipid-binding pocket found in the Galectin-1 and Galectin-3 proteins that are critical for its function, stability and membrane localization. Liza Aizman presented *in vivo* studies using FTS in autoimmune diseases models. Using an *in vivo* glioma model in mice with intact immune systems, she argued that FTS may regulate the inflammatory response of the tumor, thereby providing further therapeutic benefits on top of the growth inhibitory effects. Viki Makovski studied FTS as a potential therapeutic agent in lymphangioleiomyomatosis (LAM). Mutations in the TSC2 complex, which are found in some LAM cases, result in hyper activation of the Rheb-GTPase that is modified by a farnesyl, hence, making it a candidate target of FTS. Indeed, FTS treatment reduces active Rheb, cell migration and proliferation in LAM cellular model and is therefore a possible therapeutic agent for this rare disease. Ran Levy presented surprising results that suggest that Galectin-3 regulates K-Ras levels and activity not only via protein–protein interactions, but also by regulation of a miR. Nir Rainy and Assaf Grunwald used advanced microscopic techniques to study Ras transfer, which occurs between cells through nanotubes, and within cells through Rasosomes (random moving nanoparticles). Roni Rak and More Sela presented novel strategies for identifying new mutant p53-binding partners and new non-ribosomal peptides.

Henning Walczak (London, UK) is studying the details of the TNF-$\alpha$ receptor complex as a model system to learn more about molecular mechanisms involved in receptor-mediated apoptosis. He hypothesized that the specifics of the spatio-temporal recruitment events to the receptor are achieved by different ubiquitination patterns. Indeed, linear ubiquitination, a new form of ubiquitin chains, is critical for orchestrating the TNF-$\alpha$ receptor complex formation. Silvia Prieske (Walczak group) presented surprising findings on the consequence of TRAIL stimulation leading to activation of non-apoptotic caspase-dependent processes.

Cristina Muñoz-Pinedo (Barcelona, Spain) is interested in targeting tumor cells by taking advantage of their deregulated glucose metabolism. She described the effect of the glycolysis inhibitor 2-deoxyglucose on sarcoma cell lines. Surprisingly, apoptosis induced by 2-deoxyglucose was associated with ER stress, emphasizing the importance of glucose usage for glycosylation in tumor cells.

Seamus Martin (Dublin, Ireland) highlighted an unusual function for oncogenic Ras as an inducer of cell death. Intriguingly, the inducible ectopic expression of oncogenic Ras in human ovarian epithelial cells induces caspase-independent cell death, which was found to be autophagic cell death, yielding a new angle on Ras signaling and control of autophagy.

Poul Sorensen (Vancouver, Canada) discussed how cells with activated oncogenes need to modify key signaling and metabolic pathways in order to adapt to the stress conditions of the tumor environment. More specifically, he reported that the translation regulator YB-1 reprograms tumor cells to promote a more aggressive phenotype. Importantly, this trait could be a target for novel therapeutics. In a series of elegant experiments, Gabriel Leprivier showed how eEF2 kinase links translation elongation and survival of tumor cells in response to stress. Mads Daugaard is studying Hace1, a tumor

suppressor discovered in the Sorensen laboratory. He proposed a model to explain how the loss of this protein in mice promotes tumor formation by increasing oxidative stress, thereby linking Hace1 to the ROS generating cellular machinery. Barak Rotblat reported on how the tumor suppressor Hace1 regulates the cellular response to oxidative stress by promoting the anti-oxidant response. Interestingly, he proposed a link between this function and the protective role of Hace1 in neurodegeneration.

The Villunger group (Innsbruck, Austria) presented their recent work on mouse models to understand the physiological function of apoptosis-related proteins. Andreas Villunger gave an overview of the effects of Bmf knock out in mice focusing on its role in the remodeling of the mammary gland and in lymphocyte development. Eleonora Ottina presented her results on transgenic mice with Tet-regulated knock down system in the hematopoietic compartment. She showed how reduction of factors that are essential for granulopoiesis impairs the DN3/DN4 transition and causes pre-B-cell accumulation. Lukas Peintner used a Burkitt lymphoma model to show that specific components of the PIDDosomes have tumor suppressor activities, while other components exhibit tumor-promoting activities. Florian Bock showed that PDCD5 binds to TIP60 in response to DNA damage, highlighting the importance of PDCD5 for apoptosis in response to genotoxic stress.

Michael Bergmann (Vienna, Austria) is developing influenza viruses that are engineered to stimulate the expression of modulators of the immune response to selectively kill cancer cells. Sebastian Dorn (Volker Wacheck group, Vienna, Austria) is developing new viral vectors to improve the efficiency of current vectors. Rudolf Oehler (Vienna, Austria) identified proteins of the complement system that correlated with response to therapy by using proteomic analysis of serum samples from cancer patients. Balasz Hegedus (Vienna, Austria) demonstrated, by using expression analysis, that spheroid cultures mimic the biophysical properties of a solid tumor, offering exciting opportunities for future studies of cancer cell biology. Karin Nowikovsky (Vienna, Austria) found that LETM1 is essential for potassium extrusion from the mitochondria. These findings may have implications in Wolf-Hirschhorn syndrome, which harbors a defect in this system.

The meeting was concluded with a so-called 'pool seminar', wherein the organizer wrapped up the presented data in the form of a poem at the swimming pool.

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